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NUTRITIONAL STATUS, ANEMIA AND ASSOCIATED FACTORS IN CHILDREN AND ADOLESCENTS ATTENDING PUBLIC SCHOOLS IN THE NORTHEAST OF BRAZIL: A BEFORE AND AFTER INTERVENTION WITH IRON SUPPLEMENTATION

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ABSTRACT

OBJECTIVE: To assess nutritional status and factors associated with iron deficiency in children and adolescents, before and after intervention with iron amino chelate. **METHODS:** In this community-based, double-blind, randomized controlled trial, we assessed children aged 3-14 years. Children were cluster randomized to receive either iron supplemented food (intervention), or the same food without supplement (control). **RESULTS:** Most participants presented adequate height-for-age and BMI-for-age values. No association between nutritional status and the iron deficiency anemia (IDA), independently of the allocation group, was observed. After intervention, there was a reduction of anemic children in both groups; however there was a more significant reduction in the intervention group when compared to the control group, p<0.0001. There was also a greater reduction in the number anemic individuals with low height-for-age in the intervention group when compared to control, p=0,028. Those who remained anemic during the study were more likely to present short stature at the end, with no association or difference between the two groups. **CONCLUSIONS:** It was concluded that in the supplementation group that those who remained anemic during the trial were more likely to continue anemic after intervention, with shorter stature; with no other association or difference between groups.

KEYWORDS: Iron deficiency anemia; Nutritional status; Obesity; Children; Adolescents.

INTRODUCTION:

The etiology of iron deficiency (ID) results from the depletion of reserves in the body and may occur due to several factors, among them being physiological, nutritional and pathological.[1] In addition to the aspects considered as aggravating, low socioeconomic and cultural levels, basic sanitation conditions and access to health services directly reflect on the intake of food and, consequently, nutritional status.

The most recent data available on the nutritional status of the Brazilian population is in the Family Budget Survey (FBS).[2] These results confirmed that our population has been gaining weight in a worrisome manner. This trend was observed in 1989, when, for the first time, we were able to accompany a downward trend in malnutrition and the beginning of the escalation of excess weight.

One in three Brazilian children aged between 5 and 9 years are overweight, being 34.8% for boys and 32% for girls, and obesity rates are 16.6 and 11.8%, respectively, according to the FBS (2010).[2] The Cardiovascular Risk Study in Adolescents (ERICA) (2015)[3] reports that 8.4% of Brazilian adolescents are obese, and 25.5% of adolescents aged 12 to 17 years are overweight.

These results lead us to prioritize, with certainty, the prevention and fight against excess weight; however, some considerations are pertinent showing that Brazil, with its regional inequalities, hunger and malnutrition are still a cause for concern. Between 1989 and 2006, the northeast region was the one that most advanced in the reduction of malnutrition among children, from 9.6 to 2.2% in weight deficit, and a reduction from 32.9 to 5.8% for height deficit.[3]

Although, in 2014, Brazil officially left the UN hunger map after reaching rates of less than 5%, the lack of food is still a reality in specific regions of this South American nation; 7.2 million Brazilians face a serious situation of food deprivation. This means that one in four Brazilian households still reports some degree of food insecurity.[4]

The northeast is the region with the highest rate of households suffering from food insecurity, where 38.1% of the households present some type of food restriction.[5]. Indigenous populations are also the greatly affected by food deficit; while chronic malnutrition reaches 40% in indigenous children, this rate is 7% for the rest of the country, which means that indigenous children are twice as likely to die before their first birthday than other Brazilian children, for reasons that could be avoided.[6]

The prevalence of height deficit and anemia constitute one of the most important indicators to express inadequate nutritional conditions of populations.[6] However, their relationship has not been definitively established, although anemia might negatively affect the food intake of children and adolescents.

In this manner, the aim of the present study was to evaluate nutritional status and its relationship with anemia, in a before and after intervention, with several regional food preparations supplemented with iron amino chelate compared to control, in the context of a community intervention, where both groups received educational actions in nutrition and health.

METHODS:

Study design:

This was a community-based, double-blind, randomized controlled trial conducted with children and adolescents between the ages of three and fourteen years, of both genders, enrolled in municipal public schools in Cabo de Santo Agostinho, in the state of Pernambuco, Brazil. All the public schools in the municipality served the population with a low socioeconomic level.

The selection process was conducted using a table of random numbers. Once the list of schools was established (ten elementary schools), contact with the institutions was made to clarify the objectives and methods of the project.

Sample size was calculated considering the values of the hemoglobin (Hb) difference between the intervention and control groups, between the before intervention (Time 0, T^0) and after intervention (Time 1, T^1), by analysis of variance for repeated measures with the variable transformed into ranks, setting the level of significance at 5% and a power of 80%. The minimum number of participants in each group was 80 (n=160 participants); thus, the power of the sample was 99.9%.

A total of 3,000 Free Prior and Informed Consent forms were sent to parents or guardians, which were signed, authorizing the participation of 2,353 students. The study followed established criteria and was approved by the Research Ethics Committee of the Universidade Federal de São Paulo / Escola Paulista de Medicina (UNIFESP/EPM), under protocol #1040/07.

Exclusion criteria were: the presence of hematological or chronic diseases; already using iron supplements; history of blood transfusion (in the last three months); presence of obvious bleeding, or chronic or repetitive diarrhea; presence of malformations that significantly alter body mass and/or height measurements; Hb concentration <9.5 g/dL prior to intervention.[7]

Intervention:

Using a table of random numbers, five schools were selected to receive food with the iron-supplemented diet, constituting the intervention group (IG); the other five schools received the same diet, but without the supplement, constituting the control group (CG). Two supervisors at a local kitchen, where all the food destined to the schools of the municipality was prepared and distributed, were trained in the application of the iron dosages in the diverse alimentary preparations.

The eligible population was considered as those who consumed the standard portion of the food preparations offered, following the quota per capita established according to age group (minimum of 100 g and maximum of 240 g), determined

by the group of nutritionists in charge of the School Food Service in the municipality .

A pilot project with duration of 90 schooldays was used to evaluate the initial proposed dosage of 2.0 mg of iron per 100 g of ready-to-eat food in the intervention. However, in this period, it was noticed that the dosage did not present significant results; therefore, the dose was increased to 4.0 mg per 100 g for the study.

The study lasted for 180 schooldays, and biochemical evaluations were performed at two moments: before and after intervention. Mean iron dose was 4.0 mg per 100g of the ready-to-eat food in the intervention group; the control group received the same preparations but without iron supplementation.

Fasting blood samples (5.0 ml) were obtained at the school by means of peripheral venous puncture, and sent to the central laboratory for analyzes in the municipality, being processed on the same day, in order to determine the hemogram using a hematology analyzer (Abbott Cell Dyn 1800 Hematology Analyzer - GMI Inc., Ramsey, USA).

The criteria used for the diagnosis of anemia were according to the World Health Organization;[7] in other words, children under the age of five years with serum Hb levels below 11.0 g/dL, 11.5 g/dL for children aged five to eleven years, and 12.0 g/dL for those twelve to fourteen years of age were considered anemic, and severely anemic when Hb levels were below 9.5 g/dL.

In order to characterize the nutritional status of the study population, body mass (weight) measurements in kilograms (kg) were collected using a digital electronic scale (Aura 807 Digital Personal Scale – Seca, Hamburg, Deutschland) with a 150 kg capacity in 100 g graduations; and height in centimeters with a mobile stadiometer (Mobile Stadiometer 213 I – Seca, Hamburg, Deutschland) with 1 mm graduations. Subsequently, Body Mass Index (BMI) and the height-for-age ratio were calculated. Measurements of body mass and height were performed following the technique proposed by Jellife (1966).[8]

Data on height, body mass and age were used to calculate the percentile of height-for-age and body mass-for-age ratios. To interpret the results, the new growth curves proposed by the World Health Organization were used as reference.[9] Anthropometric data were analyzed using Anthro and AnthroPlus software, version 1.0.2.[10]

Statistical analysis:
For descriptive statistics, the absolute and relative frequency, mean and standard deviation were used to describe age. For analytical statistics, the McNemar and marginal homogeneity tests were used (exact, when necessary) for the comparisons of the frequencies between times. For this, only the individuals with data collected before and after intervention were kept in the analyses. In order to compare the groups at T^0 and the association between the maintenance of the anemia state and the anthropometric variables at T^1, the chi-square test (with Yates correction or for linear trend, when necessary) and the exact test of Fisher were used. To compare the mean age between the groups, Student's t-test was used for independent samples with homogeneous variances. The level of significance was set at 5% ($p \leq 0.05$). The statistical software package SPSS for Windows, version 15.0, was used for all analyses (SPSS Inc., Chicago, IL, USA).

RESULTS:
The total sample consisted of 1,651 children and adolescents, 52.2% of whom were male. Mean age was 8.8±2.3 and 9.0±2.5 years, respectively, in the intervention (n=878) and control (n=773) groups. There was no statistically significant difference in age and gender between the two groups.

When observing the nutritional status at T^0, it was seen that the majority of the children showed adequate values of height-for-age and BMI-for-age. There was no evidence of anthropometric differences between the groups (Tables 1 and 2).

Table 1. Nutritional status of children <5 years of age in each group, according to the study period, in the municipality of Cabo de Santo Agostinho - PE, Brazil.

Variables	Intervention group			Control group		
	T^0	T^1	$p^‡$	T^0	T^1	$p^‡$
Height-for-age (percentile)			†			†
< 3	-	-		-	-	
≥ 3	30 (100.0)	23 (100.0)		39 (100.0)	33 (100.0)	
BMI-for-age (percentile)			0.50			1.00
< 3	-	-		1 (2.6)	-	
≥ 3 and ≤ 85	23 (76.7)	18 (78.3)		28 (71.8)	22 (66.7)	
> 85	7 (23.3)	5 (21.7)		10 (25.6)	11 (33.3)	

All numbers are absolute except numbers in brackets, which represent percentages

‡ Based on McNemer or marginal homogeneity tests (T^0 vs. T^1)

† Insufficient data to calculate p-value

Table 2. Nutritional status of children and adolescents ≥5 years and <20 years of age in each group, according to the study period, in the municipality of Cabo de Santo Agostinho - PE, Brazil.

Variables	Intervention group			Control group		
	T^0	T^1	$p^‡$	T^0	T^1	$p^‡$
Height-for-age (percentile)			1.00			0.79
< -2	98 (11.6)	91(11.1)		70 (9.5)	69 (10.0)	
≥ -2	750 (88.4)	727 (88.9)		664 (90.5)	621 (90.0)	
BMI-for-age (percentile)			0.14			0.31
< 3	40 (4.7)	45 (5.5)		32 (4.4)	33 (4.8)	
≥ 3 and ≤ 85	657 (77.5)	634 (77.5)		537 (73.2)	498 (72.2)	
> 85	151 (17.8)	139 (17.0)		165 (22.5)	159 (23.0)	

All numbers are absolute except numbers in brackets, which represent percentages

‡ Based on McNemer or marginal homogeneity tests (T^0 vs. T^1)

There was no association between the nutritional status of the children and the condition of iron deficiency anemia (IDA) at T^0, regardless of the allocation group. On the other hand, there was a higher percentage of anemic individuals with low height-for-age in the IG than in the CG (11.4% vs. 5.7%). After intervention (T^1), there was a reduction of anemic children in both groups; however there was a more significant reduction in the IG when compared to the CG, from 255 to 31 and 212 to 66, respectively, p<0.0001 (Fisher's exact test). There was also a greater reduction in the number anemic individuals with low height-for-age in the IG than in the CG, from 29 to 8 and 12 to 12, respectively, p=0,028 (Fisher's exact test) (Tables 3 and 4).

Table 3. Nutritional status of children and adolescents in each group, according to the presence of iron deficiency anemia at T0, in the municipality of Cabo de Santo Agostinho – PE, Brazil.

Variables	Intervention group		p*	Control group		p*	$p^§$	$p^‡$
	Anemic	Non-anemic		Anemic	Non-anemic			
Height-for-age (percentile)			0.66			0.31	0.05	0.46
< 3 or <-2	29 (11.4)	46 (10.0)		12 (5.7)	31 (8.3)			
≥ 3 or ≥-2	226 (88.6)	413 (90.0)		200 (94.3)	343 (91.7)			
BMI-for-age (percentile)			0.54†			0.87†	0.23†	0.03†
< 3	14 (5.5)	19 (4.1)		7 (3.3)	15 (4.0)			
≥ 3 and ≤ 85	188 (73.7)	361 (78.6)		154 (72.6)	269 (71.9)			
> 85	53 (20.8)	79 (17.3)		51 (24.1)	90 (24.1)			

All numbers are absolute except numbers in brackets, which represent percentages

* Intra-group p-value (anemic vs. non-anemic)

§ Inter-group p-value comparing anemic participants

‡ Inter-group p-value comparing non-anemic participants

† p-value based on chi-square test for linear trend (exact, when necessary)

Table 4. Nutritional status of children and adolescents in each group, according to the presence of iron deficiency anemia at T^1, in the municipality of Cabo de Santo Agostinho - PE, Brazil.

Variables	Intervention group		p*	Control group		p*	p§	p‡
	Anemic	Non-anemic		Anemic	Non-anemic			
Height-for-age (percentile)			0.009			0.003	0.49	0.94
< 3 or <-2	8 (25.8)	65 (9.5)		12 (18.2)	34 (6.7)			
≥ 3 or ≥-2	23 (74.2)	622 (90.5)		54 (81.8)	475 (93.3)			
BMI-for-age (percentile)			0.85†			0.09†	0.38†	0.03†
< 3	3 (9.7)	38 (5.5)		1 (1.6)	23 (4.5)			
≥ 3 and ≤ 85	22 (71.0)	529 (77.0)		49 (74.2)	363 (71.3)			
> 85	6 (19.3)	120 (17.5)		16 (24.2)	123 (24.2)			

All numbers are absolute except numbers in brackets, which represent percentages

* Intra-group p-value (anemic vs. non-anemic)

§ Inter-group p-value (intervention vs. control) comparing anemic participants

‡ Inter-group p-value (intervention vs. control) comparing non-anemic participants

† p-value based on chi-square test for linear trend (exact, when necessary)

When analyzing the final nutritional status considering the remission of IDA, in the IG, it was observed that those who remained anemic during the study were more likely to present low height at the end of the intervention. No other associations were found nor were there differences between the groups (Table 5).

Table 5. Nutritional status of children and adolescents in each group, according to the maintenance of iron deficiency anemia between T^0 and T^1, in the municipality of Cabo de Santo Agostinho - PE, Brazil.

Variables	Intervention group		p*	Control group		p*	p§	p‡
	Anemic at T^0 Anemic at T^1	Anemic at T^0 Non-anemic at T^1		Anemic at T^0 Anemic at T^1	Anemic at T^0 Non-anemic at T^1			
Height-for-age (percentile)			0.05			1.00	0.60	0.15
< 3 or <-2	6 (24.0)	23 (10.0)		3 (6.3)	9 (5.5)			
≥ 3 or ≥-2	19 (76.0)	207 (90.0)		45 (93.7)	155 (94.5)			
BMI-for-age (percentile)			0.72†			0.72†	0.48†	0.36†
< 3	3 (12.0)	11 (4.8)		1 (2.1)	6 (3.7)			
≥ 3 and ≤ 85	16 (64.0)	172 (74.8)		35 (72.9)	119 (72.6)			
> 85	6 (24.0)	47 (20.4)		12 (25.0)	39 (23.7)			

All numbers are absolute except numbers in brackets, which represent percentages

* Intra-group p-value

§ Inter-group p-value (intervention vs. control) comparing anemic participants at T^0/T^1

‡ Inter-group p-value (intervention vs. control) comparing anemic participants at T^0/non-anemic participants at T^1

† p-value based on chi-square test for linear trend (exact, when necessary)

DISCUSSION:

The initial evaluation of nutritional status revealed that the most frequent deficit in the population under the age of 10 years was height-for-age (14.4%) and BMI-for-age (5.1%). It was also observed that anemia was associated in both the IG and CG with short height, with no difference between the groups. This fact can be explained, in large part, because it is a phase of life in which the growth rate is intense and without the necessary input of macronutrients and micronutrients, stature is the first affected marker.

School-age height reflects the health and nutritional status of populations in the most vulnerable period of physical growth. However, in Brazil, height deficit fell from 29.3% in 1974-1975 to 7.2% in 2008-2009 among boys, and from 26.7% to 6.3% in girls. The literature identifies that both male and female children and adolescents, from low-income families, have a shorter stature than those with higher income.[11-13]

It should also be noted that change has occurred in the nutritional profile of the Brazilian and Latin American population in recent decades, in all social strata.[11,14]

According to the Brazilian FBS,[2] one in three children aged 5 to 9 years was overweight. As for male adolescents, overweight prevalence increased from 3.7 (1974-1975) to 21.7% (2008-2009), while this prevalence among females rose from 7.6 to 19.4%, respectively.

In a cross-sectional study of children and adolescents aged 5 to 19 years, the prevalence of short stature and excess weight/obesity was 9.1 and 24%, respectively. Excess weight and stature deficit were higher in 15-year-old adolescents; in relation to gender, malnutrition was similar and excess weight affected the female gender more.[15]

Recent studies demonstrate a possible causal relationship between ID and BMI; this relationship identifies that individuals with low iron levels are more likely to develop obesity, in other words, increased inflammatory activity in the obese adipose tissue may favor the production of hepcidin, which at high concentrations negatively regulates iron output in macrophages and duodenal enterocytes, reducing circulating iron and favoring anemia.

The coexistence of IDA and obesity could apparently represent a paradox, since the former would be associated with nutritional deficiencies and the latter with excesses. However, recent findings suggest that obesity may predispose to IDA, evidencing a possible relationship between these health disorders.

This occurs because ferroportin is the receptor for hepcidin, and the hepcidin-ferroportin interaction controls iron levels in enterocytes, hepatocytes, and macrophages. The hepcidin-ferroportin complex is internalized in the basolateral membrane domains of the macrophages and ferroportin is degraded, blocking the release of iron from these cells. As a consequence, iron accumulation occurs in the hepatocytes and macrophages, and a reduction of the iron to plasma flow results in low transferrin saturation, reducing the amount of iron released for erythroblast development. In this manner, iron regulates the secretion of hepcidin, which in turn controls the concentration of ferroportin on the surface of cells. Hence, hepcidin is considered the main regulator of dietary iron absorption and the release of cellular iron. Therefore, if an increase in hepcidin occurs in obesity, ID may also occur. Furthermore, ID in obese individuals has been reported by other researchers,[16,17] even among obese postmenopausal women.[18]

For a long time, the association between obesity and IDA has been explained through an unbalanced diet in the individuals affected by these two nutritional problems.[19,20] However, recent findings in the fields of physiology and molecular biology have raised the discussion that obesity per se, not just diet, could be the predisposing factor for the development of IDA in different individuals.[16]

As early as in the 1960s, the first epidemiological studies showed a higher proportion of ID among obese individuals.[21] Subsequently, this relationship was also observed by several other authors, as much in adults as in children and adolescents.[22] In the present study, the result found did not indicate a relationship between anemia and excess weight or obesity, but when it compared non-anemic children and adolescents at T^1 from both groups, those belonging to the CG had a higher prevalence of excess weight and a lower prevalence of normal weight than non-anemic children and adolescents from the IG.

The higher prevalence of anemia among overweight and obese individuals has also been reported among adults. In a review of data from the first edition of the National Health and Nutrition Examination Survey (NHANES-I),[23] Micozzi, Albanes and Stevens (1989)[24] found that high BMI values were associated with low serum iron concentrations in women and that transferrin saturation was significantly lower in the highest BMI quartile, in both men and women.

While information on the prevalence and the independent consequences that IDA and obesity may bring to individuals and societies seems abundant, on the other hand, there is still a lack of prospective research, particularly at a national level, on the relationship between the two conditions, and on the harm that the combination of these two nutritional problems may cause. Anemia can be characterized as a nutritional deficiency, independent of malnutrition.[25]

The present study, like those of other authors, did not associate severe malnutrition with low levels of Hb. This fact can be explained by the fact that they present common risk factors, such as inadequate diet, poor access to health and hygiene in the physical environment, and a low level of family education.

The phase of adolescence is characterized by rapid growth and the acquisition of adult phenotypes. During this period, iron needs are elevated through the expansion of blood volume, due to the increase in lean mass in boys and at the onset of menarche in girls. ID may impair physical endurance, immune response, temperature regulation, energy metabolism and cognitive performance.[26]

Most national and international surveys are conducted with pregnant women and children less than 6 years of age, since they are highly vulnerable populations. Therefore, studies with school-age populations are relatively rare in the Brazilian community. In addition, a control group was used to strengthen the conclusions of the investigation and avoid bias.

CONCLUSIONS:

Throughout the world there is concern about the nutritional deficiency of iron and the search for efficient ways to overcome it. These contributions have, in fact, been widening the discussion, and allowing more and more advance in research using community fortification and supplementation programs. The findings of this study implicate in recommending the monitoring of child growth and Hb status of children and adolescents in order to reduce health damage and minimize severity at subsequent ages.

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Conflicts of Interest:

None of the authors have any conflicts of interest.

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